Sciencia Acta Xaveriana
An International Science Journal
ISSN. 0976-1152

Vol. 1 No. 2
pp. 51–58
Sep. 2010

Spore Forming Bacterial Biofertilizer for Phosphate Solubilization and Bio-control Agent

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Abstract. Spore forming bacteria are resistance to heat, drying, radiation, chemicals, disinfectants and antibiotics. So, the spore forming bacteria are used as a biofertilizer in agricultural purposes. Microbial solubilization of hardly soluble mineral phosphate in soil is an important process. Phosphorus involved in photosynthesis, respiration, energy storage and transfer, cell division and enlargement etc. Ten soil samples were collected from different rhizosphere regions and spreaded on Pikovaskaya's agar medium and PDA for isolation of Phosphate solubilising bacteria (PSB) and pathogen (**Fusarium**). The isolated PSB of **Bacillus Species** was taken for further studies such as micro morphological and cultural characteristics and antagonistic activity against **Fusarium**. The strain improvement was carried out on Tricalcium Phosphate (TCP) agar, different temperature, pH, UV treatment and antibiotic sensitivity test. The field soil sample was analyzed and pot culture method was carried out. The spore forming **Bacillus** strain is resistant to various environmental factors. It improves the crop production and controls the plant pathogen in groundnut.

Keywords: Phosphate solubilising bacteria, Biocontrol

(Received: 06 May 2010)

1 Introduction

Phosphorus is one of the major limiting factors for crop production on many tropical and subtropical soils as a result of high phosphorus fixation [5]. The inorganic forms of phosphorus in soil are compounds of calcium, iron, aluminium and fluoride. The organic forms are compounds of phytins, phospholipids and nucleic acids. The phosphobacteria have the ability to solubilize the insoluble forms of phosphates into soluble form with the help of organic acids such as acetic, lactic, propionic, fumaric, isovaleric, isobuytric, gluconic etc [8], vitamins and growth promoting substances like indole acetic acid (IAA) and gibberllic acid (GA3) which are secreted by the genera of **Bacillus, Pseudomonas, Rhizobium** [7] to plant which can't absorb insoluble form of phosphorus. Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plants. A large number of diseases attack Ground nut in India [2]. Fungi cause the majority of plant disease and several of them reduce yield in certain regions and seasons. Among the soil-borne fungal disease of groundnut with disease caused by **Fusarium** is potential threat to groundnut production and is of considerable economic significance for groundnut grown under irrigated condition. This disease causes severe damage and cause yield loss over 25% [4].

2 Materials and methods

The soil samples were collected from different rhizosphere region of agricultural field soil and plant root soil. The samples were serially diluted and 0.1ml of sample was spreaded on Pikovaskaya's agar medium and PDA medium for isolation of phosphate solubilizing bacteria (PSB) and **Fusarium**. The PSB culture was collected separately,

sub cultured and stored in refrigerator. The isolated PSB were inoculated into Pikovaskaya's broth and incubated at 50 °C for 3–5 days for spore formation and it was identified by endospore staining, morphology, cultural and biochemical tests and named as PSB1 to PSB3.

2.1 Determination of antagonistic property

The isolated PSB strains were determined for antagonistic activity. The cultures were inoculated to nutrient broth and incubated at 28 °C for 4–5-days. The culture filtrates were transferred to PDA medium at 5 ml filtrate per 100 ml of medium and poured to a sterilized petridish. The mycelium of **Fusarium** was grown on PDA medium at 28 °C. Agar discs of 0.7 cm were removed from the activity grows in petridish culture and placed in the center of petridishes containing PDA supplemented with filtrates. Averaging 2 perpendicular mycelial diameters substrating 0.7 cm of initial disc diameter radial growth of **Fusarium** was measured on 5-th day after inoculation measured the growth rates. In strain development, Tricalcium phosphate agar and Pikovaskaya's agar was prepared and the strains were streaked on the plates and incubated at 37 °C for 24–48 h to identify the acid production and phosphate solubilization. The MHC plate was used to identifie antibiotic resistance. The potential strains were grown on Pikovaskaya's media with different range of pH 3, 7 and 9 and 0.1ml was spreaded on the plates and expose to ultra violet light for 6 minutes (without lid).The pot soil was analyzed using physio-chemical parameter such as Texture, Limesatus, Nitrogen, Phosphorus concentration and electrical conductivity for the field study of plant growth promotion

2.2 Mass culture of Bacillus species

The isolates were inoculated in pikovaskaya's broth seperately and incubated for 24–48 h for log phase culture. Pot culture assay of plant growth and promoting activity of antagonistic isolates such as **Bacillus** species grown on pikovaskaya's agar for 4 to 6 days. The surface sterilized healthy looking groundnut seeds were inoculated with 5ml of separate culture filtrates and allow to 3–5 h for inoculation. After treatment the seeds were sown (3 seeds/pot) in 15 cm diameter of five clay pots containing sterilized sand and red soil (2:1 ratio) and 1ml of appropriate culture filtrate was added. Above the treatment was carried out by the following treatment.

A—Control, **B**—PSB1, **C**—PSB2, **D**— PSB3. Moisture level of the soil was maintained by adding sterilized water. After 60 days the plant height and number of leaves were determined. The ANOVA approach was used to evaluate the growth of groundnut in pot soil.

3 Results and discussion

The initial phosphate solubilizing ability was identified in 10 strains in Pickovaskaya's medium by a clear halo zone and plant pathogen **Fusarium** on PDA plates shows cottony pink colour appearance. Among the 10 strains, 3 strains formed endospores and shows gram positive rod shaped motile in nature. The spore forming isolate was named as PSB1, PSB2, and PSB3 and used for strain improvement. The phosphate solubilization and acid production indicate clear zone around the colonies. The PSB3 is resistant to erythromycin, ampicillin and vancomycin. It shows best growth against U.V. rays and pH at neutral (7.0), moderate growth in acid (3.0) and alkaline pH (9.0). In soil analysis the physical parameter of pot soil shows medium lime status, sandy clay

loom texture and acidic pH (8.7). Chemical parameter indicate 59 kg/acre of nitrogen, 4.0 kg/acre of phosphorus i.e., low level in availability, 96 kg/acre of potassium i.e., medium level in availability and 0.18 dcm^{-1} of electrical conductivity. The antagonistic property of PSB against growth of **Fusarium** (mm) on culture filtrate indicate that PSB1 (14.0), PSB2 (09.0) and PSB3 (4.0). The PSB3 highly inhibit the growth of **Fusarium** The plant growth promoting activity indicates that the control (Without inoculums) shows 3.5 cm in shoot length and 8 no. of leaves. The PSB1 shows 4.5 cm in shoot and length and 10 no. of leaves. The PSB2 shows 5.5 cm in shoot length and 18 no. of leaves. The PSB3 strain shows 8.0 cm in shoot length and 30 no. of leaves against control 3.5 cm in shoot length and 8 no. of leaves. The phosphobacteria persist in unfavorable condition by the formation of endospore. It's found in soil [9]. The crop requires phosphates, soluble phosphate fertilizer used for crop improvement [3]. The applications of **Bacillus** were inhibit the growth of **Fusarium** in wheat. The spore forming **Bacillus** have ability to solubilize phosphate as well as control the Fungal pathogen **Fusarium** even in unfavorable condition.

Table 1: Growth of Fusarium (mm) on Antagonistic culture filtrates (Bacillus) enriched medium.

S. no.	Isolated strains	Growth of Fusarium (mm)
1.	Control	24.0
2.	PSB1	14.0
3.	PSB2	9.0
4.	PSB3	4.0

The ANOVA approach gives F value (633.51) is grater than tabulated value of F distribution (99.42). The null hypothesis is not accepted and alternative hypothesis is

Table 2: Influence of antagonistic culture filtrates (Bacillus) on shoot length (cm) and no. of leaves.

Isolated strain	Shoot length	No. of Leaves
Control	3.5 ± 0.07	8 ± 1.45
PSB1	4.5 ± 0.05	10 ± 1.58
PSB2	5.5 ± 0.27	18 ± 0.78
PSB3	8.0 ± 0.31	30 ± 1.13

accepted.

So, $H_1 : \mu_1 \neq \mu_2 \neq \mu_3$.

Therefore the mean of sample I, II and III are not equal.

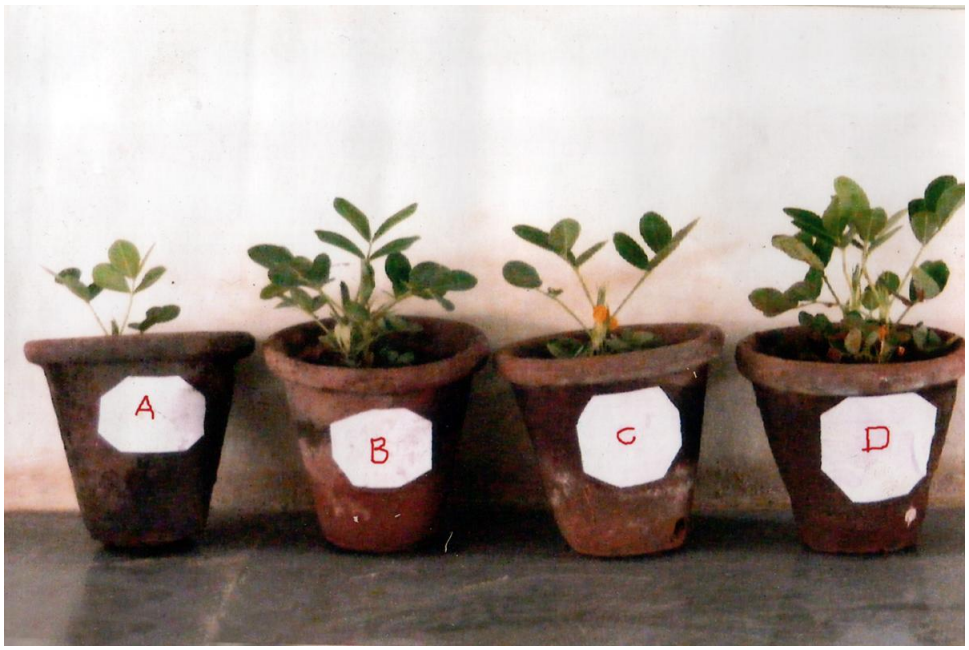

Figure 1: Plant growth promoting activity. Pot culture essay Phosphate Solublizing Bacterial Strain: A. Control, B. PSB1, C. PSB2, D. PSB3.

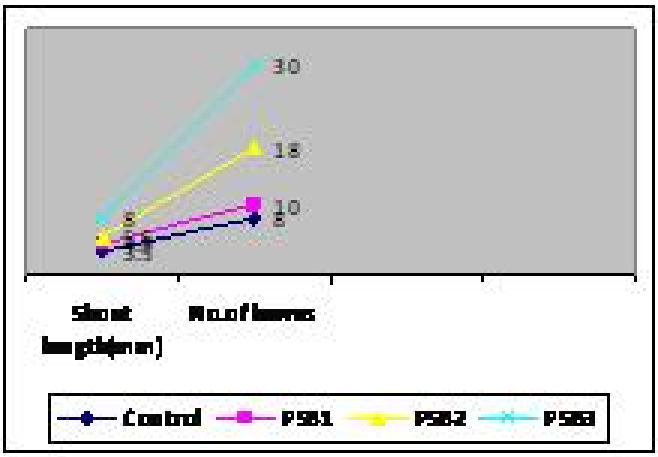

Figure 2: Influence of antagonstic filtrate on shout length and number of leaves.

Acknowledgement

We are grateful to our Principal Prof. L. Kuppusamy and Vice-Principal Prof. S. Uvarajan and the management of Indo-American College, Cheyyar, for providing us all facilities to carry out this research work in the department.

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